

FORUM

The Utility of an International Sera Bank for Use in Evaluating the Potential Human Allergenicity of Novel Proteins

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INTRODUCTION

In the safety assessment of novel foods produced through biotechnology, careful consideration is given to determining the allergenic potential of newly introduced proteins. IgE serum screening is one tool for evaluating whether the protein in question has sequence identity to a known allergen or if the source of the gene encoding the protein is a known allergenic food. A “specific” serum screen involves testing a gene product with sera from patients with documented clinical allergy to a specific allergen to confirm that the gene product of interest is not the same protein to which the patient produces IgE antibodies. A “targeted” serum screen involves testing the gene product of interest with sera from patients sensitive to food or aeroallergens from the same broad group. The concept of a global sera bank with accessible, well-characterized sera for use in such assays is an appealing option. This paper summarizes the consensus elements from a workshop to evaluate the potential utility of an international sera bank for evaluating the allergenicity of novel proteins. Areas of agreement following the workshop included the following: (1) specific sera screens are appropriate for exploring potentially cross-reactive proteins that have been identified through bioinformatics analyses; however, additional validation is needed, particularly for targeted sera screens, (2) practical and ethical considerations may preclude the formation of a global sera bank, and therefore, (3) a regional network of clinicians who could serve as sources of patient sera or be approached to conduct sera studies would be the most practical alternative.

Key Words: sera bank; allergenicity; serum screening; atopic diseases.

In recent years, there has been a considerable amount of attention given to evaluating the safety of foods produced through genetic modification using biotechnology. Foods that have been produced using biotechnology have been transgenically modified to achieve a number of characteristics including insect and herbicide resistance and nutritional enhancement. Such modifications result in the introduction of a new or “novel” protein into the food. As a result of these genetic modifications, there is a need to comprehensively evaluate the risk and safety associated with foods that have been modified using biotechnology. A fundamental part of evaluating the risk and safety associated with these foods is an allergenicity evaluation of the newly expressed protein in the modified food product.

Evaluating the potential allergenicity of novel proteins has historically relied on a “weight-of-evidence” approach that considers a number of factors including the source of the novel protein, the similarity of the amino acid sequence of the novel protein to that of known allergens, and the stability of the novel protein to heat and simulated gastric fluid. In cases where the amino acid sequence of the novel protein was similar to that of a known allergen, serum screening has been used to assess the IgE reactivity of patients who have documented clinical allergy to food allergens which have a similar amino acid sequence to the novel protein.

In agreement with a previous recommendation (Metcalf *et al.*, 1996), FAO/WHO (2000) suggested that specific serum screening be conducted if the source of the novel gene was a known allergenic food or aeroallergen and sera from patients with that specific allergy would be used to confirm that the gene product of interest was not the protein to which the patient produced IgE antibodies. FAO/WHO further suggested that

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a targeted serum screen be conducted in all other cases. *Codex Alimentarius* Commission (2003) elected not to support the recommendations for targeted serum screening because its usefulness had not been practically demonstrated. Furthermore, the utility of serum screening in the absence of sufficient structural similarity between the protein of interest and a known allergen as recommended by Thomas *et al.* (2005) (e.g., at a level of 35% over 80 or greater amino acids) has not been rigorously tested.

The availability of serum from well-characterized (i.e., clinically relevant) allergic patients is essential for successful serum testing. Thus, there would be substantial utility for an international serum bank, which could be used for novel protein safety testing. Accordingly, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) Protein Allergenicity Technical Committee organized a workshop in April 2006 to discuss the potential utility of an international sera bank for evaluating the allergenicity of novel proteins with sera from food allergic patients. The purpose of this paper is to summarize the workshop discussions associated with the development of an international sera bank for evaluating novel proteins. These discussions included a review of patient diagnostic criteria for inclusion in a sera bank, the appropriate application of serum testing, the logistical and management issues associated with serum banks, and the use of *in vitro* and *in vivo* testing to make decisions about the inclusion of sera in a bank.

FOOD ALLERGY—CLINICAL BACKGROUND, ATOPIC INDIVIDUALS, AND DIAGNOSTIC CRITERIA FOR INCLUSION IN A SERA BANK

Atopic diseases such as food allergy, asthma, and atopic dermatitis (AD) are characterized by IgE sensitization to foreign proteins. Atopy refers to an individual being prone to develop allergic disease due to a genetic state of hyper-responsiveness to mount IgE responses to common allergens. A food allergy is a specific immunologic reaction to an otherwise harmless food component, and the majority of these reactions are classified as a type I hypersensitivity reaction that is mediated by IgE antibody. The classical clinical symptoms of IgE-mediated food allergy, local reactions such as swelling of the tongue or oral mucosa, or systemic reactions such as urticaria or vomiting appear within minutes to 2 h following each ingestion of the offending substance. The skin, the gastrointestinal tract, respiratory tract, and cardiovascular system are the primary target organs in food allergy (Sicherer, 2000). The oral allergy syndrome is the most frequent clinical presentation of food allergy seen in adult patients (Mari *et al.*, 2005). A minority of food allergy reactions are cell mediated, rather than IgE mediated, but both are distinct from food intolerances which are not immune mediated.

Food allergy affects up to 8% of children and 2–4% of adults (Osterballe *et al.*, 2005; Zuberbier *et al.*, 2004). However, the

prevalence of food allergy is estimated to be much higher by the general population (perceived vs. true food allergy symptoms). Therefore, an accurate clinical history of consistent allergic reactions to the food, coupled with carefully selected allergy tests that support the history, is essential to identify appropriate patients whose sera would be included in a sera bank. The gold standard for definitively diagnosing food allergy is a double-blind, placebo-controlled food challenge (DBPCFC): allergy skin prick tests and IgE Radio Allergosorbent Tests (RASTs) only suggest the diagnosis of food allergy.

AD is a chronic inflammatory skin disease that affects up to 10% of children. Approximately 30–40% of children with moderate to severe AD also have food allergy that has been confirmed with DBPCFC. For most patients with AD, the total serum IgE and allergen-specific IgE levels may be markedly elevated, but exposure to the allergens often does not result in clinical symptoms. These patients have asymptomatic sensitization and must be differentiated from those with true allergy for the purposes of sera-screening studies. Only sera from patients who demonstrate classical symptoms of food allergy and who have positive allergy tests to the food in question should be included in sera banks used to evaluate novel proteins. The sera is further designated as “specific” if it is used to test the allergenicity of novel proteins derived from the “same” food or a cross-reacting protein to which the serum donor was allergic, not merely sensitized. In contrast, “targeted” serum testing evaluates the allergenicity of novel proteins derived from foods “similar” (i.e., to the same broad group) to the food causing the serum donor’s food allergy.

APPLICATIONS OF SERUM SCREENING

Two case studies where a limited targeted serum screen was employed using a bank composed of sera from Danish allergic patients provide practical examples of the application of serum screening. The first case examines the potential allergenicity of the Nangai nut (*Canarium indicum*) prior to allowing import into Europe (Poulsen, 2004; Sten *et al.*, 2002). In this case, a targeted sera screen was performed using sera from patients allergic to pollen (from mugwort, birch, grass, etc.) or cat or dog dander. The results showed positive IgE reactions by using several tests. For instance, 13% of the patients tested showed IgE specificity to Nangai nut by histamine release assays and 17% showed IgE specificity to RAST. In addition, open food challenges were performed, with 3 out of 12 patients tested with Nangai showing a positive response. The Nangai nut was classified as a presumptive allergen based on these *in vitro* and *in vivo* tests. However, using DBPCFC tests, this could not be confirmed in two patients. As it turned out, the proposal to import the Nangai nut was abandoned because of other food safety problems, specifically mycotoxin contamination. In the second case study, a microbial transglutaminase isolated from the organism *Streptovorticillium mobaraense* had

a 5 contiguous amino acid identity to the major codfish allergen, heat stable allergen Gad c1. Among the 25 fish allergic sera, no binding between patient IgE and transglutaminase was observed (Pedersen *et al.*, 2004).

Therefore, although sera showing positive reactions *in vitro* to a food or an inhaled allergen may be used for studying the degree and the character of cross-reactivity between a known allergen and a novel protein, it is suggested that only sera from clinically reactive patients (i.e., well-characterized sera) are actually used to perform risk evaluations. Other classes of sera may still be interesting and relevant for outlining the type of cross-reactivity between a known allergen and a novel protein; however, they may contain IgE antibodies of low biological activity or low clinical relevance. In these examples, the targeted serum screen did not add significant value to the allergy safety assessment. Consequently, the utility of targeted sera screens needs to be further investigated and validated before it is broadly used in the safety assessment of novel proteins.

SERA BANKS

Different organizations around the world have developed or proposed the development of sera banks containing the sera from allergic patients. There appears, however, to be different motivations for developing these sera banks such as (1) to increase the understanding of food allergies, (2) to better understand the prevalence and distribution of specific allergies, (3) to predict whether the inclusion of a novel protein or novel food in the food supply will result in the development of new allergies or whether the protein will be cross-reactive with existing allergens, (4) to identify specific allergens, and (5) for use in postmarketing surveillance strategies to monitor the potential for development of reactivity to newly introduced foods. These different motivations impact the design of the sera bank and especially the selection of patients whose sera are to be included.

Arguably, the most advanced regional sera banks are located in Europe and South Korea. The goal for the European (i.e., EuroPrevall) Serum Bank is to investigate the prevalence and distribution of food allergies throughout Europe in infants, children, and adults. More specifically, the project intends to determine the threshold doses for different allergenic foods, investigate the role of the environment in determining the different patterns of food allergy, measure the socioeconomic impact of food allergy, and develop new diagnostic tools to provide a better correlation of *in vitro* diagnostic results with the clinical situation. The EuroPrevall Sera Bank has strict criteria for inclusion of patient sera. For example, six different case record forms, including information on clinical history, skin prick tests to 42 different commercial extracts, and food challenge studies must be completed for each patient. There are plans to evaluate approximately 70,000 subjects within the different substudies of EuroPrevall. The EuroPrevall project

involves 20 mostly European countries and 54 partners. A key element in this project is the development of a clinical databank with standardized reporting and collection of data allowing clinical patterns to be identified.

The Korean Sera Bank was developed to facilitate population monitoring for reactivity to commercially available products produced through biotechnology (i.e., postmarketing surveillance) as well as to evaluate products seeking approval using targeted and specific sera-screening methods. The Korean Sera Bank is being constructed in two phases involving 10 branch centers throughout Korea. In the first phase, sera from patients with allergy symptoms to a variety of sources (e.g., house dust, pollen, mold, food including egg white, milk, soy, corn, wheat, tomato, potato, rice, sesame, red pepper, etc.) that gave a positive skin test or reacted positively in a CAP Fluorescent Enzyme Immunoassay were included. Confirmation of the clinical reactivity of the serum donors by challenge tests has not been performed in the majority of cases. Currently, 1434 sera samples have been collected with a subset of 237 sera available for use in monitoring the development of allergies in response to exposure to products produced through biotechnology. In the second phase, the sera will be further classified, although details on the further classification of the sera had not been developed.

As noted above, not only are the purposes of the two sera banks different but also are the criteria for inclusion of sera in the two sera banks different, with the EuroPrevall Sera Bank having more stringent criteria for sera inclusion than that required for the Korean Sera Bank. To maximize the potential for informing the safety assessment of novel proteins, sera used in such studies should have significant clinical documentation. The "gold standard" for inclusion of patient sera is clinical reactivity confirmed by DBPCFC. In lieu of DBPCFC, which is not always possible to conduct, criteria for serum inclusion should include a convincing history of clinical allergic reactions to a specific allergen. In the case of anaphylactic reactions where challenges cannot be performed, clinical reactivity can be evaluated using IgE-binding studies and biological activity assays (e.g., histamine release assays; skin prick tests) (Table 1).

Although a sera bank would be useful to track the development and prevalence of allergies over time and to study the properties of food allergens, the development of a centrally located sera bank may be impractical for a variety of reasons. There are many unresolved questions regarding the funding and maintenance of such a bank, what would happen to the sera in the bank if funding was eliminated (i.e., who owned the sera?), and who would have access to such sera. There are also ethical questions related to the use of the sera for research and commercial purposes. Often, sera are obtained for research purposes exclusively and are restricted from being used for commercial purposes. There are further concerns around which clinical allergies should be represented in the bank (e.g., should they be regionally based, ethnically based, include allergy to just the major food allergens or be more broadly based to include all foods, aeroallergens, and insect venoms, i.e., like those

TABLE 1
Diagnostic Criteria for Including Sera in a Bank
(in order of preference)

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1. Clinical reactivity confirmed by DBPCFC
 2. Convincing clinical history of reaction to specific allergen
 3. IgE-binding studies or biological activity assays
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contained in allergen databases?). Ultimately, a sera bank should contain clinically documented sera from as many known allergens as possible, but the above-mentioned practical and ethical concerns would make it extremely difficult to implement.

The formation of a network of clinicians from each region of the world who could be used as a resource for identifying sera and conducting sera studies would be a reasonable alternative to developing an international sera bank.

IGE TESTING—HOW TO USE *IN VITRO* AND *IN VIVO* TESTING TO MAKE DECISIONS ABOUT INCLUSION OF SERA

Sera from allergic patients are of interest because of the allergen-specific IgE molecules they contain. They can be used as reagents in various analyses with proteins of interest to make an assessment of potential cross-reactivity. However, while specific IgE is necessary to cause allergic disease, it is not sufficient to trigger the allergic cascade unless at least two IgE-binding epitopes are available within the allergen. Additionally, there must be high avidity between the IgE and the epitope, sufficiently abundant IgE, and exposure to the allergen in order to elicit a clinical food allergic reaction.

The biological versus clinical relevance of IgE-binding assays is critically important. Distinguishing allergen-specific IgE from total IgE is relatively easy, while it is much more difficult to distinguish clinically relevant IgE binding from specific IgE binding with little or no correlation to clinical symptoms. For instance, a high frequency of IgE binding to fruits, nuts, and vegetables is observed in birch pollen allergic subjects without food allergy (see, e.g., Reuter *et al.*, 2006) or to wheat in grass pollen allergic subjects without food allergy to cereals (Jones *et al.*, 1995). This lack of clinical relevance of many positive IgE-binding tests may be explained by the fact that the biological activity of an IgE molecule is mainly determined by the avidity of the allergen-specific IgE and by the epitope valency. In practice, these antibodies are at the basis of many false positive results for food allergy in diagnostic tests. On the contrary, the absence of IgE binding to a given food has a high negative predictive value, provided that a high-quality allergen preparation is used for testing.

IgE test methods include *in vitro* assays such as dot blot (microarray), immunoblot (may be done under reducing, non-reducing, native, or two-dimensional conditions), Enzyme-Linked ImmunoSorbent Assay, basophil histamine release

assays, RAST, EAST, or inhibition assays (Poulsen *et al.*, 2006). Skin prick tests and DBPCFC are examples of *in vivo* IgE-binding methods. The IgE tests must be validated in terms of sensitivity of IgE detection (detection limit) and isotype specificity (e.g., cross-reactivity of the detection system with IgG) and general method performance characteristics such as inter- and intra-assay variation. They must include positive and negative control sera and must include positive and negative control proteins and extracts. Serum donors must be selected with relevant, clinically proven allergies. Validation of the IgE test establishes reproducibility from day to day, analyst to analyst, and among different extract preparations. A limit of detection should be established as well as a limit of quantitation and positive cutoff values. There is not a clear consensus regarding which assay is preferable for sera characterization. However, the more physiologically relevant methods, such as histamine release assays or basophil activation tests, provide greater insight into whether IgE reactivity of a serum would be of clinical significance.

CONCLUSION

The use of sera banks to facilitate specific serum screens is scientifically defensible, but there are practical limitations because these screens only detect antigen-specific IgE binding, which is not necessarily representative of clinically relevant allergic response. The targeted serum screen is more controversial as a result of its lack of validation and unknown utility for risk assessment and safety testing.

Specific serum screens may also play a role in exploring potentially cross-reactive proteins that have been identified through bioinformatics analyses when they utilize well-characterized patient sera. However, the utility of serum-screening tests, particularly targeted serum screens, for risk and safety evaluations should be further investigated and validated. While the concept of sera banks that could supply clinically well-documented patient sera for testing the potential allergenicity of novel proteins is attractive, there are practical and ethical considerations that may preclude this from becoming reality. Consequently, the formation of a network of clinicians from each region of the world who could be utilized to conduct sera studies or, alternatively, would serve as sources of sera from appropriately characterized patients would be the most beneficial and best path forward.

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